DP Barcode: 459470 MRID No.: 51159001

DATA EVALUATION RECORD
ACUTE (SINGLE DOSE) TOXICITY TESTS WITH THE HONEY BEE LARVAE
NON-GUIDELINE

1. **CHEMICAL:** Acetochlor PC Code No.: 121601

2. **TEST MATERIAL:** Acetochlor Technical Purity: 96.6%

3. **CITATION**

Authors:	Tomé, H.V.V. et al.
Title:	Acetochlor Technical: An Acute Larval Toxicity Study with the Honey Bee (*Apis mellifera*)
Study Completion Date:	October 18, 2019
Laboratory:	Eurofins EAG Agroscience, LLC Easton, MD
Sponsor:	Sharda Cropchem Ltd. Mumbai, India 662H-101
MRID:	51159001
DP Barcode:	459470

4. **REVIEWED BY:** Julie Burns, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: Julie Burns **Date:** 12/18/2020

REVIEWED BY: Elizabeth Krupka, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: Elizabeth Krupka **Date:** 12/30/2020

5. **REVIEWED BY:** Meghann Niesen, Ecologist, EFED, ERB5

Signature: Digitally signed by MEGHANN NIESEN
Date: 2021.04.13 09:15:24 -04'00'

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

6. STUDY PARAMETERS

Test Species and strain: Honey bees (*Apis mellifera*)
Age of Test Organisms at Test Initiation: 1st instar larvae, 72-hours post-hatch
Exposure Duration: 48 hours

7. CONCLUSIONS:

Individual synchronized honey bee (*Apis mellifera*) larvae (newly hatched) were exposed *in vitro* to Acetochlor Technical on Day 4 of the study at the nominal dietary concentrations and doses reported in the table below. Measured diet concentrations and dietary doses were calculated by the study author based on the analyzed diet samples and are also summarized below.

Nominal Concentration (mg ai/kg diet)	Measured Concentration (mg ai/kg diet)	Nominal Dietary Dose (µg ai/larva)	Measured Dietary Dose (µg ai/larva)
183	181	6.3	6.19
366	359	13	12.3
732	716	25	24.5
1460	1438	50	49.2
2920	2871	100	98.2

Larvae used in the study were obtained from the test facilities' own supply (Alachua, FL). A negative control was run, and dimethoate (purity: 98.3%) was used as a reference toxicant at 257 mg ai/kg diet, corresponding to a nominal dose of 8.8 µg ai/larva. All groups consisted of 3 replicates with 12 larvae/replicate for a total of 36 larvae per treatment and control group, placed within 48-well cell culture plates. Each of the three separate colonies was considered to be a replicate.

After 72 hours, there was an absence of mortality in the negative control and lowest test level. Mortality ranged from 3 to 97% in the four highest test levels, with mortality increasing notably from one level to the next in the three highest test levels. On Day 7, unconsumed diet was noted in 3, 0, 6, 13, 71, and 100% of surviving bees in the negative control and measured 6.19, 12.3, 24.5, 49.2, and 98.2 µg ai/larva treatment groups, respectively. The dimethoate treatment resulted in 61% mortality. Based on these results, the 72-hr LC_{50} was determined to be 1316 mg ai/kg diet, and the 72-hr LD_{50} was 45.0 µg ai/larva.

The study **is scientifically sound** and is classified as **supplemental** for measuring acute (single dose) toxicity to honey bee larvae.

	Mortality
Diet Concentration (mg ai/kg diet)	LC_{50}: 1316 95% CI: 1127 - 1528 Slope: 5 (3 - 6)
Dietary Dose (µg ai/larva)	LD_{50}: 45.0 95% CI: 38.5 - 52.3 Slope: 4.8 (3.4 – 6.3)

8. ADEQUACY OF THE STUDY

A. Classification: This study **is scientifically sound** and is classified as **supplemental (quantitative).**

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS: The test was performed in accordance with the OECD Guidance Document 237 on Honey bee (*Apis mellifera*) Larval Toxicity Test, Single Exposure (2013). The following deviations were noted:

1. The replicates in this experiment were defined as a colony consisting of 12 bees. Individual bee data were not reported as recommended by the EPA's Honeybee Toxicity Testing Frequently Asked Questions (August 16, 2018) where a replicate is defined as the individual bee present in each individual grafting cell.
2. Incomplete physiochemical properties of the test item were reported.
3. The shape of the grafted larvae at transfer was not reported. OECD recommends that newly hatched larvae are selected that have not yet formed a "C" shape.
4. It was not reported if colonies were kept in conditions conforming to proper cultural practices.
5. The % weight of yeast, glucose, and fructose are representative of the total diet weight, not the OECD recommended % of aqueous solution. Further details were not provided for the reviewer to calculate % weight of the aqueous solution for these components.
6. Raw data for environmental conditions (temperature and relative humidity) were not reported, and results were only provided as an average for the entire study period.

10. SUBMISSION PURPOSE: To determine the effects on mortality and sublethal effects of Acetochlor Technical on the honey bee (*A. mellifera* L) larvae from acute [single dose] exposure for the purpose of pesticide re-registration.

11. MATERIALS AND METHODS

A. Test Material

Test Material:	Acetochlor Technical
Description:	Light yellow liquid
Lot No./Batch No.:	20180139
Purity:	96.6%
Stability of compound under test conditions.	Analytical verification of the test item in larval Diet C samples resulted in recoveries ranging from 97% to 101% of nominal values.
Storage conditions of test chemical:	Ambient.

Physicochemical properties of Acetochlor Technical:

Parameter	Values	Comments
Molecular Weight	Not reported	
Water solubility at 20°C (mg/L)	Not reported	
Vapor pressure (torr, at 25°C)	Not reported	
Structure	Not reported	
Mean organic carbon partition coefficient K_{oc} (L/kg_{oc})	Not reported	
Log octanol-water partition coefficient Log K_{ow}	Not reported	

B. Test Organisms

Guideline Criteria	Reported Information	Comments
Species	Honey bee (*Apis mellifera*)	*OECD recommends European honey bee (Apis mellifera)*
Age at beginning of test Worker bees of uniform	Newly hatched larvae (72-hours post-hatch).	

Guideline Criteria	Reported Information	Comments
age.		*OECD recommends that on D1 of study, first instar (L1) synchronized larvae (i.e., larvae of the same age) are taken from comb of three colonies.*
Source	Test facilities' own supply (Alachua, FL). Larvae were from three different hives.	*OECD recommends larvae are collected from three different colonies.*
Were bees from disease-free colonies?	Larvae for the test were selected from brood frames collected from adequately fed, apparently healthy hives. Larvae were not treated with antibiotics, miticides, or other pesticides within the previous four weeks.	*OECD recommends that colonies used to obtain larvae should be adequately fed, health (i.e., as far as disease- and parasite-free), with a known history and physiological status.*
Were bees kept in conditions conforming to proper cultural practices?	Not reported. Larvae were from hives with a known history of apicultural practices.	

B. Test System

Guideline Criteria	Reported Information	
Test Chambers	Larvae were grafted into polystyrene cell cups (9 mm x 8 mm) containing artificial diet. Cells cups were placed in 48-well tissue culture plates and covered with lids. The grafting cell cups were positioned at the top of a cotton dental roll placed in each well. The larvae were held in a hermetically sealed plexiglass desiccator.	*OECD recommends 48-well plate with each well containing a crystal polystyrene grafting cell.*

Guideline Criteria	Reported Information	
Temperature during exposure	Average of 34.8°C. Variations in temperature were only observed when the desiccator was opened for grafting and feeding.	*OECD recommends incubator at 34 – 35°C. Deviations may occur but temperature should not be lower that 23oC or higher than 40oC; deviations not last more than 15 minutes once every 24 hrs.*
Relative humidity during exposure	Average of 92.3%. A saturated potassium sulfate solution was placed in the incubator to help maintain relative humidity. Variations in relative humidity were observed when the desiccator was opened for grafting and feeding.	*OECD recommends use of K_2SO_4 to maintain water saturated atmosphere.*
Lighting	Bees maintained in the dark except during dosing and observations.	*OECD recommends that plates should be maintained in darkness.*
Feeding	Each larva was fed once a day (except on D2) with a standardized amount of artificial diet: 20 µL untreated diet A on day 1 (D1), 20 µL untreated diet B on day 3 (D3), 30 µL treated/untreated diet C on day 4 (D4), 40 µL untreated diet C on day 5 (D5), and 50 µL untreated diet C on day 6 (D6). Unused diet C was stored refrigerated between feeding intervals. <u>Diet A*</u>: 44.25% weight of fresh royal jelly + 44.25% weight of water + 0.90% weight of yeast	*The % weight of yeast, glucose, and fructose are representative of the total diet weight, not the OECD recommended % of aqueous solution. Further details were not provided for the reviewer to calculate % weight of the aqueous solution for these components. *OECD recommends that all larvae are fed once a day. Volume of diet is adjusted each day. Additional food should be added to the cell even if previous allocation has not been totally consumed. Presence of uneaten food at termination of test should be reported.* *OECD recommends:* *Diet A (D1): 50% weight of fresh royal jelly + 50% weight of an*

Guideline Criteria	Reported Information	
	extract + 5.30% weight of glucose and 5.30% weight of fructose. Diet B*: 42.95% weight of fresh royal jelly + 42.95% weight of water + 1.30% weight of yeast extract + 6.40% weight of glucose and 6.40% weight of fructose. Diet C*: 50% weight of fresh royal jelly + 30% weight of water + 2.0% weight of yeast extract + 9.0% weight of glucose and 9.0% weight of fructose.	*aqueous solution containing 2% weight of yeast extract, 12% weight of glucose* *Diet B (D3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.* *and 12% weight of fructose.* *Diet C (from D4 to D6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose.*

C. Test Design

Guideline Criteria	Reported Information	Comments
Nominal dosage levels tested	Diet concentrations: 0 (negative control), 183, 366, 732, 1460, and 2920 mg ai/kg diet Dietary Doses: 0 (negative control) 6.3, 13, 25, 50, and 100 µg ai/bee	*OECD recommends 5 treatments of increasing test concentrations. Alternatively, when a limit test is performed, a single dose of 100 µg ai/larva or the maximum achievable solubility (whichever is lower).*
Number of bees exposed per dosage level	12 bees per replicate, with 3 replicates per test level. 36 total bees per group. Each of three different hives was considered to be a replicate.	*OECD recommends minimum of 12 larvae from each of 3 colonies allocated on the same plate to each treatment, i.e., minimum of 36 larvae per treatment.*
Other experimental	To ensure the production of	

Guideline Criteria	Reported Information	Comments
design information	uniform larvae, the queen from each hive was confined in an excluder for 24 to 26 hours on an empty frame of drawn comb in order to isolate the potential area for egg laying. After the egg-laying period, the queens were released and the frames with eggs were kept in the hive near the brood for approximately 75 hours, until the larvae hatched and reached an appropriate size for transfer. More larvae than were needed were transferred and incubated for ~48 hours.	*OECD recommends that newly hatched larvae are selected that have not yet formed a "C" shape and randomizing the allocation of larvae into the plates for each colony. On Day 1, larva is deposited in cell containing 20 µL diet.*
Bees randomly or impartially assigned to test groups	Yes, larvae were indiscriminately selected per treatment level, and well plates were indiscriminately placed on shelves within the desiccator.	*OECD recommends that each group of a minimum of 12 larvae from each of the three colonies is considered a replicate for a given treatment level and identified as such on the microplate.*
Control	Untreated diet; 36 negative control larvae.	*OECD recommends 12 larvae x 3 colonies=36 larvae minimum and that control mortality from D4 to D7 should be ≤15%.*
Solvent control	N/A	*OECD recommends maximum of 5%.*
Reference Toxicant	Dimethoate, tested at a nominal concentration of 257 mg ai/kg (8.8 µg ai/larva). 36 reference larvae	*OECD recommends technical*

Guideline Criteria	Reported Information	Comments
	exposed.	*grade dimethoate at dose of 8.8 ± 0.5 µg a.i./larva. Mortality should be ≥50% at D7 for toxic reference.*
Total observation period and frequency of interim observations	Larvae were observed daily at the time of feeding. Final observations were recorded approximately 72 hours after dosing. Observations of sublethal effects, including the presence of uneaten food were recorded on Day 7.	*OECD recommends that following chemical exposure on D4, mortalities are checked at time of feeding on D5, D6 and D7 (test termination). Other observations including presence of uneaten food on D7 should be qualitatively reported.*

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes, signed and dated statements of No Data Confidentiality, Quality Assurance, and Good Laboratory Practice Compliance were provided. This study was conducted in compliance with the GLP standards as published by the U.S. EPA (40 CFR Parts 160, 1989) and the OECD principles of GLP (ENV/MC/CHEM (98)17) with the following exceptions: periodic analyses of water, fructose, glucose, yeast extract, and royal jelly for potential contaminants; the characterization and stability of the test and reference substance in the dose solutions; and the stability, homogeneity, and verification of the reference substance in the dose solutions.
Observed adverse effects on bees at respective dosages	On Day 7, unconsumed diet was noted in 3, 0, 6, 13, 71, and 100% of surviving bees in the negative control and measured 6.19, 12.3, 24.5, 49.2, and 98.2 µg ai/larva treatment

Guideline Criteria	Reported Information
	groups, respectively.
Control and Solvent Control Mortality	Negative control: 0%
Were raw data included?	Yes. Except environmental conditions.
Analytical Analysis?	Yes, conducted using in-house using methods developed by Eurofins-Easton. Larval Diet C samples were analyzed using high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS). LOQ: 31.8 mg ai/kg diet

Mortality and Observations:

After 72 hours, there was an absence of mortality in the negative control and lowest test level. Mortality ranged from 3 to 97% in the four highest test levels, with mortality increasing with each test level. On Day 7, unconsumed diet was noted in 3, 0, 6, 13, 71, and 100% of surviving bees in the negative control and measured 6.19, 12.3, 24.5, 49.2, and 98.2 µg ai/larva treatment groups, respectively. Mortality in the positive control (Dimethoate) was 61% at test termination.

Table 1. Cumulative honey bee larval mortality data after single dietary exposure (dose).

Mean-Measured Dietary Concentration (Measured Dose)	Number Exposed	Day 5[a] (%)	Day 6[a] (%)	Day 7[b] (%)
Negative Control	36	0	0	0
181 mg ai/kg diet (6.19 µg ai/larva)	36	0	0	0
359 mg ai/kg diet (12.3 µg ai/larva)	36	0	3	3
716 mg ai/kg diet (24.5 µg ai/larva)	36	3	3	11*
1438 mg ai/kg diet (49.2 µg ai/larva)	36	17	39	53*
2871 mg ai/kg diet (98.2 µg ai/larva)	36	39	94	97*
Dimethoate (nominal) 257 mg ai/kg diet (8.8 µg ai/larva)	36	--	--	61

[a] Calculated by the reviewer in Excel using data from Appendix 6, p. 51 of the MRID.
[b] Data obtained from Table 2, p. 18 of the MRID.
--Not calculated.
*Statistically significant based on study author's results (Jonckheere-Terpstra Step-Down Test, $p < 0.05$)

Reported Statistics:

Data were assessed for normality and homogeneity of variance. Comparisons of treatment and negative control responses were performed using a Jonckheere-Terpstra Step-Down Test ($p < 0.05$). The 72-hour LC/LD_{50} with confidence limits was estimated by linear interpolation. All statistical analyses were performed using CETIS version 1.9.3.0. The study author reported the following based on the nominal concentrations and nominal doses:

NOAEC: 366 mg ai/kg diet
LOAEC: 732 mg ai/kg diet
LC_{50}: 1394 mg ai/kg diet 95% C.I.: 695.7 - 2289 mg ai/kg diet

NOAEL: 13 µg ai/larva
LOAEL: 25 µg ai/larva
LD_{50}: 47.8 µg ai/larva 95% C.I.: 23.8 - 86.1 µg ai/larva

Reviewer's Statistical Analysis:

Mortality data were analyzed using CETIS statistical software version 1.9.6.12 with database backend settings implemented by EFED on 7/25/17. Measured diet concentrations (mg ai/kg diet) and measured dietary doses (µg ai/larva) were used for the analyses and are represented in CETIS as separate test records (acronyms "dc" = diet concentration, and "dd" = dietary dose).

Treatment group mortality data were corrected for negative control mortality using Abbott's correction. The LC/LD_{50} values with 95% confidence intervals were determined using linear regression.

	Mortality
Diet Concentration (mg ai/kg diet)	LC_{50}: 1316 95% CI: 1127 - 1528 Slope: 5 (3 - 6)
Dietary Dose (µg ai/larva)	LD_{50}: 45.0 95% CI: 38.5 - 52.3 Slope: 4.8 (3.4 – 6.3)

13. REVIEWER'S COMMENTS:

The reviewer's LC/LD_{50} results were lower than the study author's. Differences can be attributed to the reviewer using linear regression results based on the measured concentrations and doses, compared to the study author using linear interpolation results based on the slightly higher values of the nominal concentrations and doses. The reviewer's results are presented in the Conclusions section of this DER, because measured concentrations/doses are the most accurate quantification of exposure.

The following validity criteria were met for the guideline followed:

1) Larval mortality from D4 to D7 in the control replicates was ≤15%.
2) In the reference chemical treatment, larval mortality should be ≥ 50% at D7.

The in-life phase of this study was conducted between July 1 and July 7, 2019.

References

Michener, Charles. 2007. Bees of the World. Johns Hopkins University Press; 2nd ed. Baltimore, MD.

Schmehl, D. R , Tomé, H. V. V., Mortensen, A. N., Martins, G. F., Ellis, J. D. 2016. *Protocol for the rearing of honey bee (*Apis mellifera *L.) workers.* Journal of Apicultural Research, 2016. DOI: 10.1080/00218839.2016.1203530.

Tidepool Scientific Software. 2011. *Users Guide, Comprehensive Environmental Toxicity Information System (CETIS).* Tidepool Scientific Software, McKinleyville, CA

OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

Batch ID:	18-0936-5044	**Test Type:**	OECD 237 Honeybee Acute Larval	**Analyst:**	
Start Date:	01 Jul-19	**Protocol:**	OECD 237: Acute Larval Single Exposure	**Diluent:**	
Ending Date:	07 Jul-19	**Species:**	Apis mellifera	**Brine:**	
Test Length:	6d 0h	**Taxon:**		**Source:**	Eurofins EAG Agroscience, **Age:**

Sample ID:	20-8497-8523	**Code:**	51159001 dc	**Project:**	Herbicide
Sample Date:	01 Jul-19	**Material:**	Acetochlor	**Source:**	Sharda Cropchem Limited
Receipt Date:		**CAS (PC):**		**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith		

121601 51159001 measured concentrations, record created by: J. Burns

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	mg ai/kg	95% LCL	95% UCL	TU	S
10-8917-0947	72h Mortality Rate	GLM: Log-Normal (Probit)		LC5	602.1	406.9	756.7		1
				LC10	715.6	516.5	871.8		
				LC25	955.1	760.9	1117		
				LC50	1316	1127	1528		
00-4867-6807	72h Mortality Rate	Trimmed Spearman-Kärber		LC50	1323	1142	1533		1

72h Mortality Rate Summary

Conc-mg ai/kg	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
181		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
359		3	0.0278	0.0000	0.1473	0.0000	0.0833	0.0278	0.0481	173.21%	2.78%
716		3	0.1111	0.0000	0.3501	0.0000	0.1667	0.0556	0.0962	86.60%	11.11%
1438		3	0.5278	0.0000	1.0000	0.1667	0.8333	0.1944	0.3368	63.81%	52.78%
2871		3	0.9722	0.8527	1.0000	0.9167	1.0000	0.0278	0.0481	4.95%	97.22%

72h Mortality Rate Detail

Conc-mg ai/kg	Code	Rep 1	Rep 2	Rep 3
0	N	0.0000	0.0000	0.0000
181		0.0000	0.0000	0.0000
359		0.0000	0.0000	0.0833
716		0.1667	0.0000	0.1667
1438		0.1667	0.5833	0.8333
2871		0.9167	1.0000	1.0000

OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

Batch ID:	19-3354-2594	**Test Type:**	OECD 237 Honeybee Acute Larval	**Analyst:**	
Start Date:	01 Jul-19	**Protocol:**	OECD 237: Acute Larval Single Exposure	**Diluent:**	
Ending Date:	07 Jul-19	**Species:**	Apis mellifera	**Brine:**	
Test Length:	6d 0h	**Taxon:**		**Source:**	Eurofins EAG Agroscience, **Age:**

Sample ID:	11-7361-5403	**Code:**	51159001 dd	**Project:**	Herbicide
Sample Date:	01 Jul-19	**Material:**	Acetochlor	**Source:**	Sharda Cropchem Limited
Receipt Date:	16 Dec-20 20:24	**CAS (PC):**		**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith		

121601 51159001 measured dietary doses, record created by: J. Burns

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	ug ai/larv	95% LCL	95% UCL	TU	S
00-4333-6155	72h Mortality Rate	GLM: Log-Normal (Probit)		LC5	20.59	13.92	25.88		1
				LC10	24.47	17.67	29.81		
				LC25	32.67	26.03	38.2		
				LC50	45.02	38.54	52.25		
03-8920-6943	72h Mortality Rate	Trimmed Spearman-Kärber		LC50	45.28	39.08	52.46		1

72h Mortality Rate Summary

Conc-ug ai/larv	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
6.19		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
12.3		3	0.0278	0.0000	0.1473	0.0000	0.0833	0.0278	0.0481	173.21%	2.78%
24.5		3	0.1111	0.0000	0.3501	0.0000	0.1667	0.0556	0.0962	86.60%	11.11%
49.2		3	0.5278	0.0000	1.0000	0.1667	0.8333	0.1944	0.3368	63.81%	52.78%
98.2		3	0.9722	0.8527	1.0000	0.9167	1.0000	0.0278	0.0481	4.95%	97.22%

72h Mortality Rate Detail

Conc-ug ai/larv	Code	Rep 1	Rep 2	Rep 3
0	N	0.0000	0.0000	0.0000
6.19		0.0000	0.0000	0.0000
12.3		0.0000	0.0000	0.0833
24.5		0.1667	0.0000	0.1667
49.2		0.1667	0.5833	0.8333
98.2		0.9167	1.0000	1.0000

OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

Analysis ID:	10-8917-0947	**Endpoint:**	72h Mortality Rate	**CETIS Version:**	CETISv1.9.6
Analyzed:	16 Dec-20 20:22	**Analysis:**	Linear Regression (GLM)	**Status Level:**	1
Batch ID:	18-0936-5044	**Test Type:**	OECD 237 Honeybee Acute Larval	**Analyst:**	
Start Date:	01 Jul-19	**Protocol:**	OECD 237: Acute Larval Single Exposure	**Diluent:**	
Ending Date:	07 Jul-19	**Species:**	Apis mellifera	**Brine:**	
Test Length:	6d 0h	**Taxon:**		**Source:**	Eurofins EAG Agroscience, **Age:**

Linear Regression Options

Model Name	Link Function	Threshold Option	Thresh	Optimize	Pooled	Het Corr	Weighted
Log-Normal (Probit)	η=inv Φ[π]	Control Threshold	0.006105	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	PMSD	F Stat	P-Value	Decision(α:5%)
59	-19.48	46.67	47.62	3.11931	0.206496	0.9959	1.61%	0.4891	0.6963	Non-Sig Lack of Fit

Point Estimates

Level	mg ai/kg	95% LCL	95% UCL
LC5	602.1	406.9	756.7
LC10	715.6	516.5	871.8
LC25	955.1	760.9	1117
LC50	1316	1127	1528

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	Test Stat	P-Value	Decision(α:5%)
Intercept	-15.11	2.337	-19.69	-10.53	-6.465	<1.0E-37	Significant Parameter
Slope	4.843	0.7456	3.381	6.304	6.495	<1.0E-37	Significant Parameter
Threshold	0.006105	0.008153	-0.00988	0.02208	0.7488	0.4540	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	6400	3200	2	2062	<1.0E-37	Significant Effect
Lack of Fit	2.536	0.8454	3	0.4891	0.6963	Non-Significant Effect
Pure Error	20.74	1.728	12			
Residual	23.28	1.552	15			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	22.43	25	0.0969	Non-Sig Heterogeneity
	Pearson Chi-Sq GOF Test	23.28	25	0.0784	Non-Sig Heterogeneity
Variance	Mod Levene Equality of Variance	1.254	4.387	0.3900	Equal Variances
Distribution	Anderson-Darling A2 Normality Te	1.152	2.492	0.0052	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.8938	0.8965	0.0448	Non-Normal Distribution
Overdispersion	Tarone C(α) Binomial Overdispersi	3.378	1.645	3.6E-04	Sig Overdispersion

72h Mortality Rate Summary

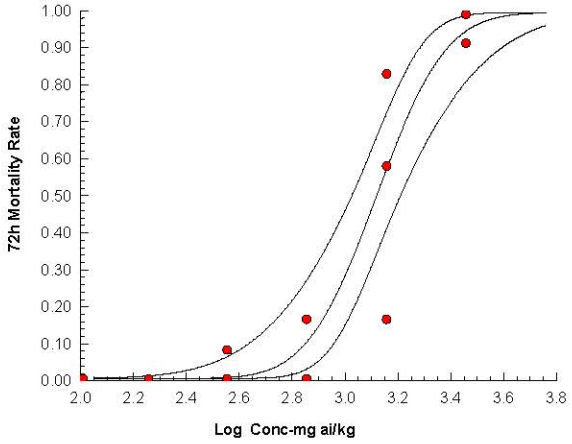
			Calculated Variate(A/B)								
Conc-mg ai/kg	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	N	3	0.0000	0.0000	0.0000	0.0000	0.0000		0.0%	0	36
181		3	0.0000	0.0000	0.0000	0.0000	0.0000		0.0%	0	36
359		3	0.0278	0.0000	0.0833	0.0278	0.0481	173.20%	2.78%	1	36
716		3	0.1111	0.0000	0.1667	0.0556	0.0962	86.60%	11.11%	4	36
1438		3	0.5278	0.1667	0.8333	0.1944	0.3368	63.81%	52.78%	19	36
2871		3	0.9722	0.9167	1.0000	0.0278	0.0481	4.95%	97.22%	35	36

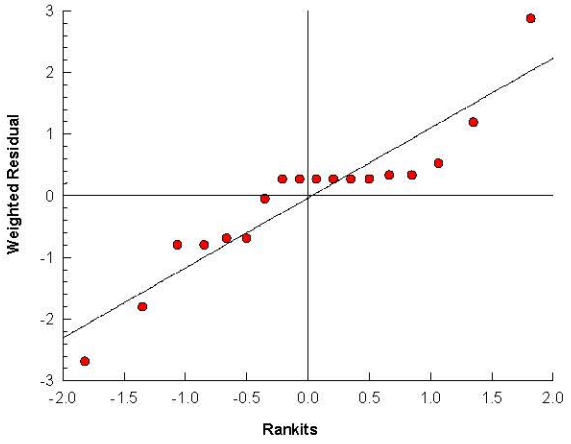
OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

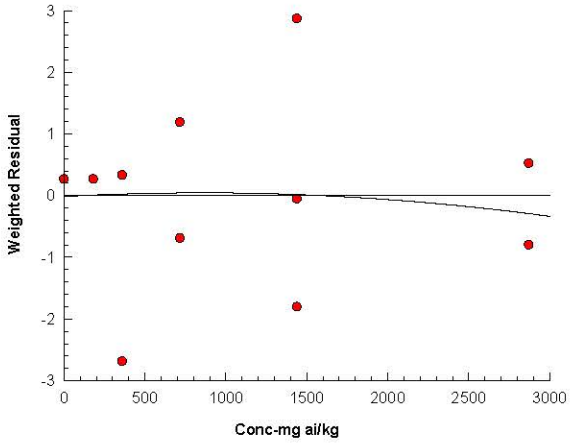
Analysis ID:	10-8917-0947	**Endpoint:**	72h Mortality Rate	**CETIS Version:**	CETISv1.9.6
Analyzed:	16 Dec-20 20:22	**Analysis:**	Linear Regression (GLM)	**Status Level:**	1

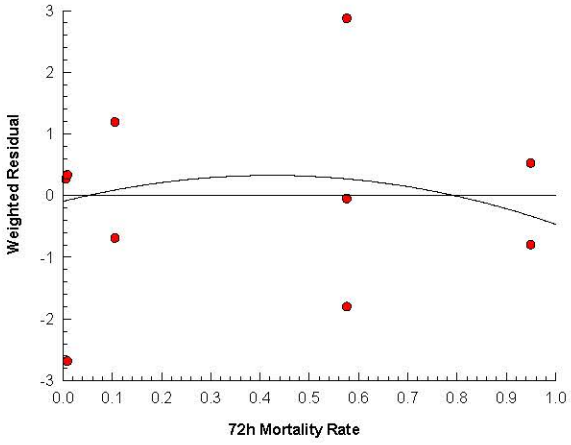
Graphics

Log-Normal: inv Φ[π]=α+β·log[x]









OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

Analysis ID:	00-4867-6807	**Endpoint:**	72h Mortality Rate	**CETIS Version:**	CETISv1.9.6
Analyzed:	16 Dec-20 20:23	**Analysis:**	Trimmed Spearman-Kärber	**Status Level:**	1
Batch ID:	18-0936-5044	**Test Type:**	OECD 237 Honeybee Acute Larval	**Analyst:**	
Start Date:	01 Jul-19	**Protocol:**	OECD 237: Acute Larval Single Exposure	**Diluent:**	
Ending Date:	07 Jul-19	**Species:**	Apis mellifera	**Brine:**	
Test Length:	6d 0h	**Taxon:**		**Source:**	Eurofins EAG Agroscience, **Age:**

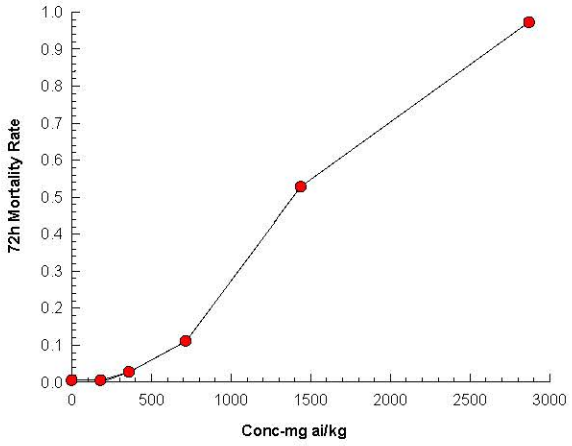
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	2.78%	3.121659	0.031984	1323	1142	1533

72h Mortality Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-mg ai/kg	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Dev**	**CV%**	**%Effect**	**A/B**	**Mean**	**%Effect**
0	N	3	0.0000	0.0000	0.0000	0.0000		0.0%	0/36	0	0.0%
181		3	0.0000	0.0000	0.0000	0.0000		0.0%	0/36	0	0.0%
359		3	0.0278	0.0000	0.0833	0.0481	173.20%	2.78%	1/36	0.02778	2.78%
716		3	0.1111	0.0000	0.1667	0.0962	86.60%	11.11%	4/36	0.1111	11.11%
1438		3	0.5278	0.1667	0.8333	0.3368	63.81%	52.78%	19/36	0.5278	52.78%
2871		3	0.9722	0.9167	1.0000	0.0481	4.95%	97.22%	35/36	0.9722	97.22%

Graphics



OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

Analysis ID:	00-4333-6155	**Endpoint:**	72h Mortality Rate	**CETIS Version:**	CETISv1.9.6
Analyzed:	16 Dec-20 20:26	**Analysis:**	Linear Regression (GLM)	**Status Level:**	1
Batch ID:	19-3354-2594	**Test Type:**	OECD 237 Honeybee Acute Larval	**Analyst:**	
Start Date:	01 Jul-19	**Protocol:**	OECD 237: Acute Larval Single Exposure	**Diluent:**	
Ending Date:	07 Jul-19	**Species:**	Apis mellifera	**Brine:**	
Test Length:	6d 0h	**Taxon:**		**Source:**	Eurofins EAG Agroscience, **Age:**

Linear Regression Options

Model Name	Link Function	Threshold Option	Thresh	Optimize	Pooled	Het Corr	Weighted
Log-Normal (Probit)	η=inv Φ[π]	Control Threshold	0.006058	Yes	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	PMSD	F Stat	P-Value	Decision(α:5%)
61	-19.48	46.67	47.62	1.653438	0.206579	0.9959	1.60%	0.4894	0.6961	Non-Sig Lack of Fit

Point Estimates

Level	ug ai/larv	95% LCL	95% UCL
LC5	20.59	13.92	25.88
LC10	24.47	17.67	29.81
LC25	32.67	26.03	38.2
LC50	45.02	38.54	52.25

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	Test Stat	P-Value	Decision(α:5%)
Intercept	-8.004	1.247	-10.45	-5.56	-6.42	<1.0E-37	Significant Parameter
Slope	4.841	0.7448	3.381	6.301	6.499	<1.0E-37	Significant Parameter
Threshold	0.006058	0.008131	-0.00988	0.02199	0.7451	0.4562	Non-Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	6445	3222	2	2076	<1.0E-37	Significant Effect
Lack of Fit	2.538	0.8458	3	0.4894	0.6961	Non-Significant Effect
Pure Error	20.74	1.728	12			
Residual	23.28	1.552	15			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	22.43	25	0.0970	Non-Sig Heterogeneity
	Pearson Chi-Sq GOF Test	23.28	25	0.0784	Non-Sig Heterogeneity
Variance	Mod Levene Equality of Variance	1.254	4.387	0.3898	Equal Variances
Distribution	Anderson-Darling A2 Normality Te	1.152	2.492	0.0052	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.8937	0.8965	0.0448	Non-Normal Distribution
Overdispersion	Tarone C(α) Binomial Overdispersi	3.378	1.645	3.6E-04	Sig Overdispersion

72h Mortality Rate Summary

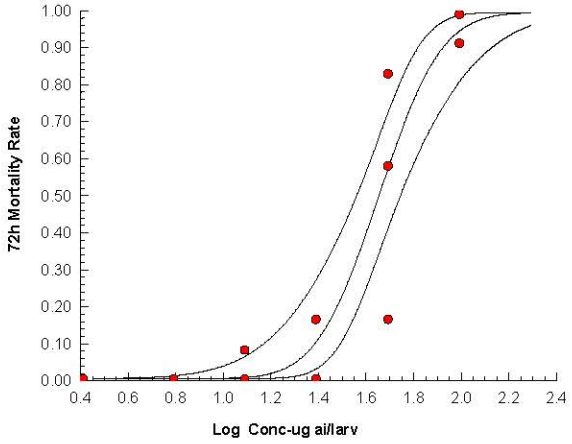
			Calculated Variate(A/B)								
Conc-ug ai/larv	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	N	3	0.0000	0.0000	0.0000	0.0000	0.0000		0.0%	0	36
6.19		3	0.0000	0.0000	0.0000	0.0000	0.0000		0.0%	0	36
12.3		3	0.0278	0.0000	0.0833	0.0278	0.0481	173.20%	2.78%	1	36
24.5		3	0.1111	0.0000	0.1667	0.0556	0.0962	86.60%	11.11%	4	36
49.2		3	0.5278	0.1667	0.8333	0.1944	0.3368	63.81%	52.78%	19	36
98.2		3	0.9722	0.9167	1.0000	0.0278	0.0481	4.95%	97.22%	35	36

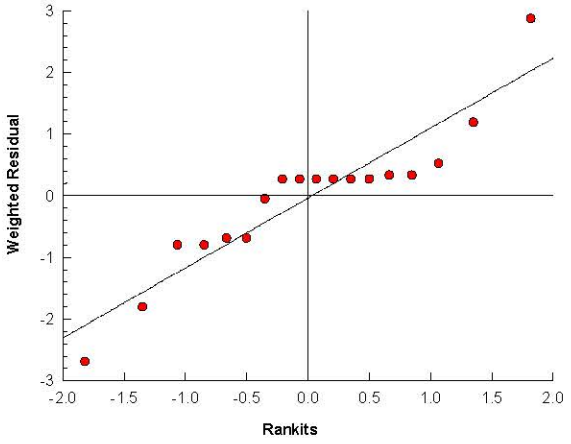
OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

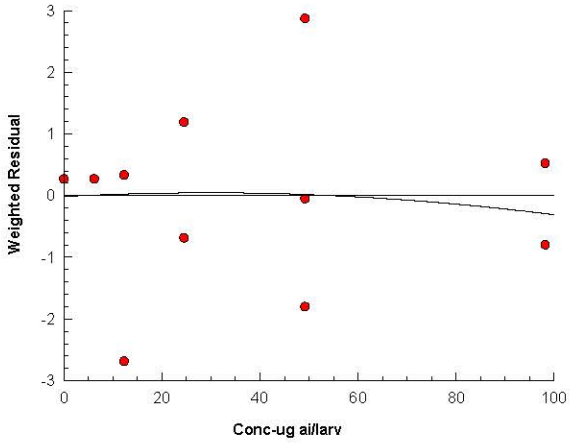
Analysis ID:	00-4333-6155	**Endpoint:**	72h Mortality Rate	**CETIS Version:**	CETISv1.9.6
Analyzed:	16 Dec-20 20:26	**Analysis:**	Linear Regression (GLM)	**Status Level:**	1

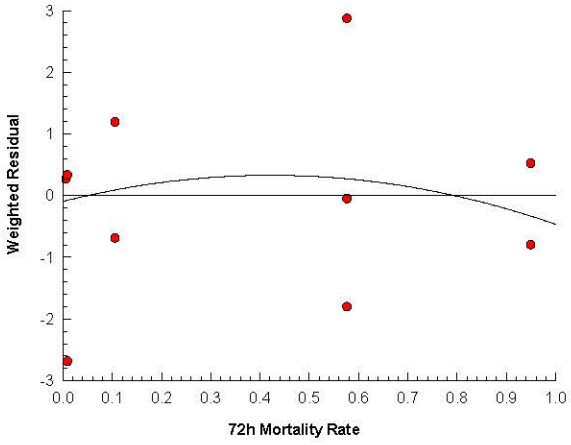
Graphics

Log-Normal: inv Φ[π]=α+β·log[x]









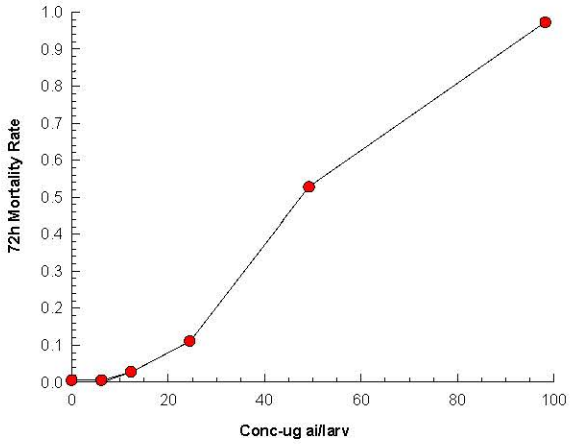
OECD TG237 Honey bee Larval Acute Toxicity — **Eurofins EAG Agroscience, LLC**

Analysis ID:	03-8920-6943	**Endpoint:**	72h Mortality Rate	**CETIS Version:**	CETISv1.9.6
Analyzed:	16 Dec-20 20:26	**Analysis:**	Trimmed Spearman-Kärber	**Status Level:**	1
Batch ID:	19-3354-2594	**Test Type:**	OECD 237 Honeybee Acute Larval	**Analyst:**	
Start Date:	01 Jul-19	**Protocol:**	OECD 237: Acute Larval Single Exposure	**Diluent:**	
Ending Date:	07 Jul-19	**Species:**	Apis mellifera	**Brine:**	
Test Length:	6d 0h	**Taxon:**		**Source:**	Eurofins EAG Agroscience, **Age:**

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	2.78%	1.655874	0.031967	45.28	39.08	52.46

72h Mortality Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-ug ai/larv	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Dev**	**CV%**	**%Effect**	**A/B**	**Mean**	**%Effect**
0	N	3	0.0000	0.0000	0.0000	0.0000		0.0%	0/36	0	0.0%
6.19		3	0.0000	0.0000	0.0000	0.0000		0.0%	0/36	0	0.0%
12.3		3	0.0278	0.0000	0.0833	0.0481	173.20%	2.78%	1/36	0.02778	2.78%
24.5		3	0.1111	0.0000	0.1667	0.0962	86.60%	11.11%	4/36	0.1111	11.11%
49.2		3	0.5278	0.1667	0.8333	0.3368	63.81%	52.78%	19/36	0.5278	52.78%
98.2		3	0.9722	0.9167	1.0000	0.0481	4.95%	97.22%	35/36	0.9722	97.22%

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